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**Supplemental Information**

**Functional Analysis of Orai1 Concatemers Supports a Hexameric Stoichiometry for the CRAC Channel**

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## SUPPORTING MATERIAL

### Functional analysis of Orai1 concatemers supports a hexameric stoichiometry for the CRAC channel

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**Table S1: PCR primers used to generate Orai1 subunit inserts**

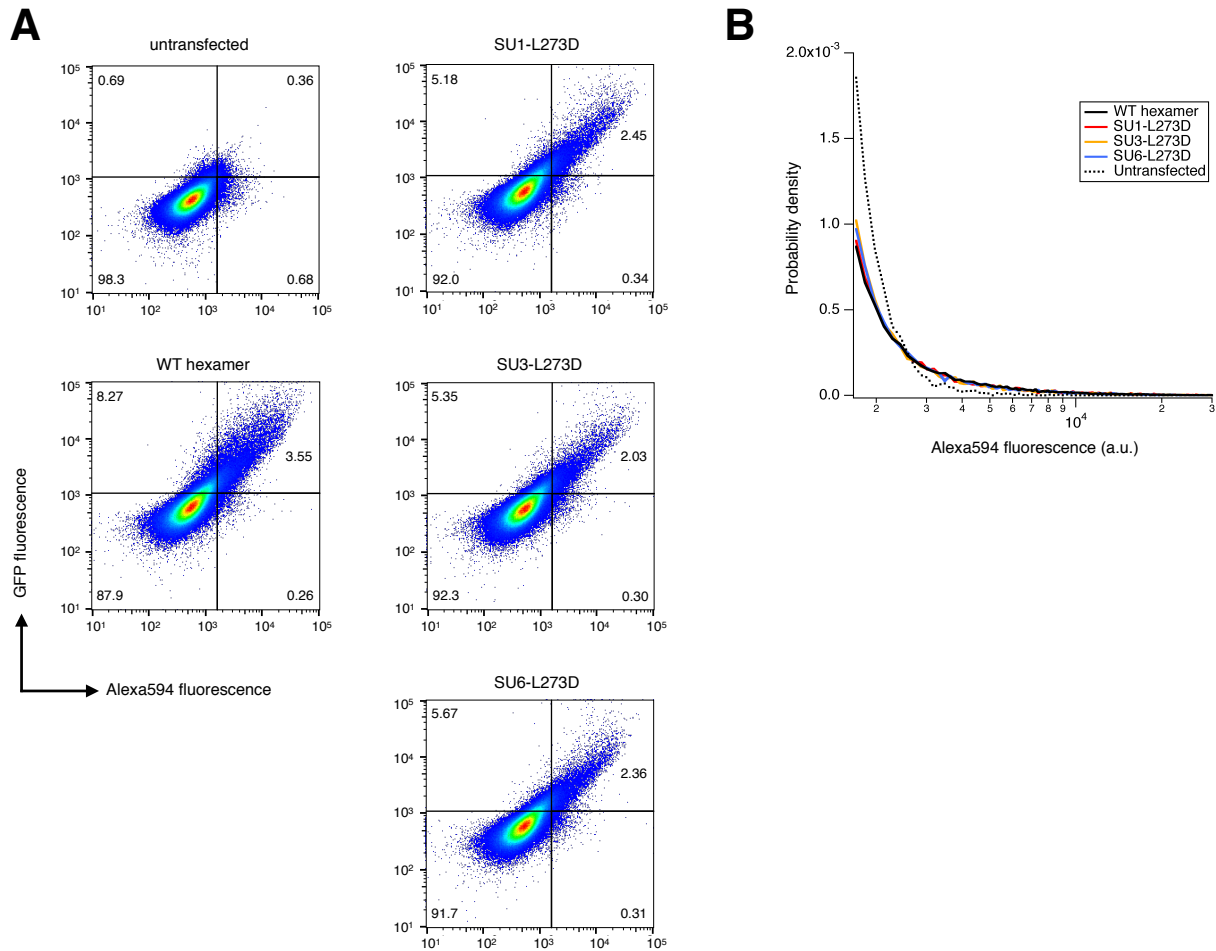
Primer	Purpose	Sequence
1	SU1 for	TCTAAGGCTCGAGGCCACCATGGAGCAAAGCTCATTTCTGAG GA
	SU1 rev	ACGGCAAGCTTGGCATAGTGGCTGCCGGGCGT
2	SU2 for	TAATAAAGCTTGAAAACCTATACTTCCAAGGAGAGCAAAGC TCATTTCTGAGGA
	SU2 rev	ACGGCGAATTCGGCATAGTGGCTGCCGGGCGT
3	SU3 for	AGGAAGAATTCGAGAACCTCTACTTCCAAGGAGAGCAAAGC TCATTTCTGAGGA
	SU3 rev	AGCAAGGTACCGGCATAGTGGCTGCCGGGCGT
4	SU4 for	AGACACGGTACCGAAAACCTATACTTCCAAGGAGAGCAAAG SU4 rev
	SU4 rev	ACGGCGACTGCAGCATAGTGGCTGCCGGGCGTCAG
5	SU5 for	AATAAACTGCAGAAAACCTATACTTCCAAGGAGAGCAAAGC TCATTT
	SU5 rev	TACGGCGATATCGGCATAGTGGCTGCCGGGCGTCAGGG
6	SU6 for	TTACTAGATATCGAAAACCTATACTTCCAAGGAGAGCAAAG SU6 rev
	SU6 rev	ATGCGTGGATCCCATAGTGGCTGCCGGGCGT
7 (SDM)	$\Delta$ Hind for	AGCCGCGCCAAGCTCAAAGCCTCCAGCCG
	$\Delta$ Hind rev	CGGCTGGAGGCTTTGAGCTTGGCGCGGCT
8 (SDM)	TRS for	GGCCCGGGATCCACGAGAACCTTTATTTCCAGGGATCGGTCGC CACCATGGTGAG
	TRS rev	ACCATGGTGGCGACCGATCCCTGGAAATAAAGGTTCTCGTGG ATCCCGGGCCCGC
9 (SDM)	L273D for	ACCGACAGTTCAGGAGGACAACGAGCTGGCGGAG
	L273D rev	CTCCGCCAGCTCGTTGTCCTCCTGGAAGTGTCTCGT

Primer sets were used for PCR amplification except where indicated. Primers used for site-directed mutagenesis are denoted by “SDM”

**Table S2: Linking sequences in the Orai1 concatemeric hexamer**

Orai1 Subunit #	N-term appended sequence	C-term appended sequence
1	XhoI-myc	HindIII
2	HindIII-linker <sup>a</sup>	EcoRI
3	EcoRI-linker <sup>a</sup>	KpnI
4	KpnI-linker <sup>a</sup>	PstI
5	PstI-linker <sup>a</sup>	EcoRV
6	EcoRV-linker <sup>a</sup>	BamHI
C-term eGFP	BamHI-TRS	

<sup>a</sup>linker = TRS (ENLYFQG) + myc (EQKLISEEDL) + Gly-Ser stretch (NGGGGS)



**Figure S1. Flow cytometry analysis of 6xOrai1 expression in the plasma membrane.** (A) Bivariate plot showing GFP (total Orai1) vs 2C1.1-Alexa594 (surface Orai1) fluorescence in untransfected HEK cells and HEK cells transfected with GFP-tagged 6xOrai1 variants. In cells transfected with 6xOrai1-GFP, GFP expression was proportional to Alexa594 fluorescence (upper right quadrants). Untransfected cells stained with 2C1.1 were used to establish a cutoff for GFP and Alexa594 negative and positive populations, as indicated by the quadrant gates. The gates were chosen separately such that 99% of untransfected cells fell in the negative population for GFP and for Alexa594. (B) Histogram of the GFP and Alexa594 double positive population in cells expressing Orai1 hexamer and untransfected cells. The Alexa594 fluorescence distribution is similar between WT and L273D hexamer populations. Each histogram contained > 1000 cells.